Immobilized Aptamer Arrays for Protein Detection and Quantification

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Aptamers, the globular oligonucleotide products of the SELEX process, have been selected for binding activity against a wide variety of targets. Affinities for proteins are generally between low pM and 10 nM. These affinities allow aptamers to be used as affinity chromatography reagents to purify dilute target proteins from complex mixtures.

Clearly aptamers could be used as capture reagents aimed at many relevant protein targets from blood or urine. Because aptamers are oligonucleotides, many of the technologies designed to create arrays of nucleic acid probes (for mRNA profiling, for example) would be entirely suitable for creating arrays of aptamers. Such arrays could be used to measure large numbers of proteins as markers of disease status.

Consideration of antibody based diagnostics suggests that specificity and sensitivity are the two criteria most critical for obtaining quantitative molecular signatures. NeXstar has developed an improvement over the standard SELEX process which should result in reagents for array-based diagnostics that are far superior to antibodies. By incorporating the appropriate modified nucleosides into the oligonucleotide library, we select aptamers which bind tightly to their target proteins, and also photocross-link to these targets at high yield. The cross-link depends on the precise positioning of a specific aptamer nucleotide with a specific amino acid in the protein. The cross-link provides a second element of specificity to the capture process, akin to a second antibody in a sandwich format. Thus cross-linking aptamers can replace both the capture and signaling components of an ELISA format, and substitute covalent interactions for non-covalent interactions. This substitution greatly facilitates the subsequent processing and analysis of the captured protein.

Measurement of the levels of proteins cross-linked to an array of aptamers can be sensitively obtained through a Universal Protein Stain. Since the capture reagents in such an array will be aptamers and not antibodies , the targets bound to their precise X,Y locations will be the only proteins on the array. Thus a Universal Protein Stain, that is, a protein-specific reagent, coupled to any desired detectable signal (fluorescence, radioactivity, biotin, etc) can be used to develop a quantitative molecular signature. Detection in appropriate formats should be straightforward.

These advancements in the use of aptamers, along with progress through genomics and research "proteomics" in the identification of disease-related marker proteins, should facilitate the use of molecular signatures in the detection and treatment of disease.